

WHAT IS CLAIMED IS:

1. A method of reconstituting a target protein in a predetermined location within an organism comprising:

5 (a) splitting DNA coding for the target protein into at least two fragments;

(b) separating the DNA fragments of step (a) to prevent transmission of the gene coding for the target protein to other organisms;

10 (c) expressing the DNA fragments of step (b) within the organism to produce the corresponding fragments of the target protein; and

(d) reconstituting the target protein from the protein fragments.

15 2. A method preventing transmission to other organisms of the gene coding for a target protein from within an organism containing said DNA coding for the target protein comprising:

20 (a) splitting DNA coding for the target protein into at least two fragments; and

(b) separating the DNA fragments of step (a) to prevent transmission of the gene coding for the target protein.

25 3. The method of claim 1 or 2, wherein the organism is selected from the group consisting of plants, animals, fungi, viruses, prokaryotes, and single-cell eukaryotes.

4. The method of claim 1 or 2, wherein the DNA coding for the target protein is split by DNA coding for one or more inteins or portions thereof.

5. The method of claim 4, wherein the DNA coding for the target protein is split by forming at least two DNA fusion fragments, wherein said DNA fusion fragments comprise a portion of the DNA coding for the target protein and a portion of DNA coding for the intein.

6. The method of claim 5, wherein one of said fusion fragments is formed by linking the C-terminal end of DNA coding for an N-terminal portion of the target protein to the N-terminal end of the DNA coding for an N-terminal portion of the intein, and another of said fusion fragments is formed by linking the N-terminal end of DNA coding for a C-terminal portion of the target protein to the C-terminal end of DNA coding for a C-terminal portion of the intein.

7. The method of claim 1 or 2, wherein the DNA coding for the target protein is split to form two or more DNA fragments by DNA coding for one or more affinity domains.

8. The method of claim 7, wherein the affinity domain is selected from the group consisting of inteins or intein fragments, leucine zipper and c-Jun/c-Fos.

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9. The method of claim 1 or 2, wherein the DNA fragments coding for the target protein are separated by compartmentalizing each DNA fragment into different compartments selected from a group consisting of the nucleus, a membrane bound organelle, a plasmid, a virus, a cosmid, and an artificial chromosome.

10. The method of claim 9, in which at least one of the DNA fragments coding for the target protein is fused to a DNA sequence encoding transit peptides such that the protein products of the DNA fragments are transported into a single compartment where functional reconstitution can occur.

11. The method of claim 10, in which one of the DNA fragments coding for a portion of the target protein is compartmentalized in the nucleus, being fused to a DNA sequence encoding a transit peptide for transport into chloroplasts, and the other DNA fragment coding for another portion of the target protein is compartmentalized in the chloroplasts.

12. The method of claim 1 or 2, wherein the DNA fragments coding for the target protein are separated by inserting each of the fragments into different portions of a DNA molecule wherein the DNA molecule is selected from the group consisting of DNA from the nucleus, a membrane bound organelle, DNA from a plasmid, DNA from a cosmid, DNA from a virus and DNA from an artificial chromosome.

13. The method of claim 12, wherein at least one of the DNA molecules is naturally inherited.

5 14. The method of claim 12, wherein at least one of the DNA molecules resides in the chloroplasts.

15. The method of claim 12, wherein at least one of the DNA molecules resides in the mitochondria.

10 16. The method of claim 4, wherein reconstitution of the target protein fragments comprises intein-mediated splicing.

15 17. The method of claim 4, wherein reconstitution of the target protein fragments comprises intein-mediated protein complementation.

18. The method of claim 1, wherein reconstitution of the target protein fragments comprises protein complementation.

20 19. The method of claim 18, wherein protein complementation occurs in the presence of an affinity domain.

25 20. The method of claim 18, wherein protein complementation occurs in the absence of an affinity domain.

21. The method of claim 1 or 2, wherein splitting of the DNA coding for the target protein comprises:

- (a) determining one or more potential split site regions of the target protein; and
- (b) splitting the DNA coding for the target protein at the potential split site region.

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22. The method of claim 21, wherein the potential split site region of the target protein is determined by analyzing primary amino acid sequence of the target protein for non-conserved regions.

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23. The method of claim 21, wherein the potential split site region is determined by linker tolerance of linker insertion within the target protein.

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24. The method of claim 21, wherein the potential split site region is determined by analyzing the structure of the target protein for the presence of flexible loops.

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25. The method of claim 21, wherein the potential split site region is determined by analyzing the structure of the target protein for the presence of amino acid sequence between folding domains of the target protein.

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26. An isolated DNA fragment comprising a DNA split site in an EPSPS gene.

27. The isolated DNA fragment of claim 26, wherein the DNA fragment is selected from the group consisting of the DNA

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32. The isolated DNA fragments of claim 26, 28, or 30, wherein said DNA fragment is fused to DNA coding for an intein or portion thereof.